INVESTOR IN PEOPLE

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

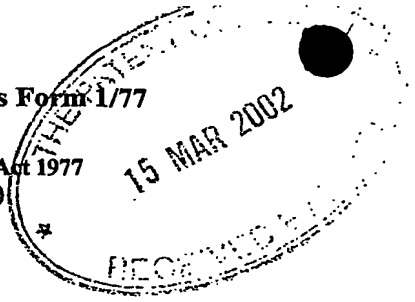
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed R. Mahoney

Dated 6 February 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)



The **Patent Office**

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP10 8QQ

~~18MAR02 E704104-1 D00524~~
P01/7700 0.00-0206215.6

1.	Your reference	4-32366P1	
2.	Patent application number *(The Patent Office will fill in this part)*	0206215.6	~~15 MAR 2002~~
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND	
	Patent ADP number *(if you know it)*		
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND	6509648904
4.	Title of invention	Organic compounds	
5.	Name of your agent *(If you have one)*		
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	B.A. YORKE & CO. CHARTERED PATENT AGENTS COOMB HOUSE, 7 ST. JOHN'S ROAD ISLEWORTH MIDDLESEX TW7 6NH	
	Patents ADP number *(if you know it)*	1800001	

6.	If you are declaring priority from one ore more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day/month/year)
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *(see note (d))*	Yes		

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	25
Claim(s)	3
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	ONE
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature: B-A-Yorke & Co — Date: **15 March 2002**

B.A. Yorke & Co.

12. Name and daytime telephone number of person to contact in the United Kingdom: **Mrs. E. Cheetham** **020 8560 5847**

Warning

After an application for a patent has been filed. the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the united Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) Once you have filled in the form you must remember to sign and date it.

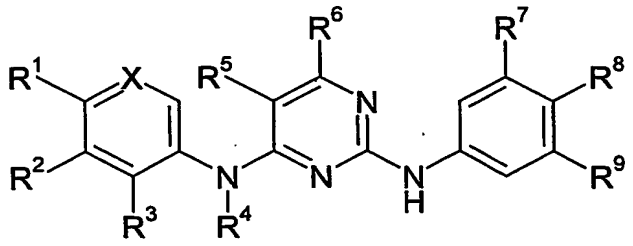
e) For details of the fee and ways to pay please contact the Patent Office.

DUPLICATE

Organic Compounds

The present invention relates to novel pyrimidine derivatives, to processes for their production, their use as pharmaceuticals and to pharmaceutical compositions comprising them.

More particularly the present invention provides in a first aspect, a compound of formula I



(I)

wherein

X is $=CR^0-$ or $=N-$;

each of R^0, R^1, R^2, R^3 and R^4 independently is hydrogen; hydroxy; C_1-C_8alkyl; C_2-C_8alkenyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkyl-C_1-C_8alkyl; C_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl which optionally may be substituted on the ring by hydroxy, C_1-C_8alkoxy, carboxy or C_1-C_8alkoxycarbonyl; or residue R^3 and R^4 form together with the nitrogen and carbon atoms to which they are attached a carbocyclic ring having 5 to 10 ring atoms or a heterocyclic ring having 5 to 10 ring atoms and comprising 1, 2 or 3 heteroatoms selected from N, O and S; or each of R^1, R^2 and R^3, independently, is halogen; halogenoC_1-C_8alkyl; hydroxy; C_1-C_8alkoxy; hydroxyC_1-C_8alkoxy; C_5-C_{10}aryl; carboxy; C_2-C_8alkoxycarbonyl; C_5-C_{10}heterocycloalkyl; C_5-C_{10}heteroaryl; nitro; C_2-C_8alkylcarbonyl; $-N(C_1$-C_8alkyl$)C(O)$ C_1-C_8alkyl; $-SO_2N(R^{10})R^{11}$; $-N(R^{10})R^{11}$; $-C_1$-C_4-alkylene-$SO_2N(R^{10})R^{11}$; $-CON(R^{10})R^{11}$; wherein each of R^{10} and R^{11} independently is hydrogen; hydroxy; C_1-C_8alkyl; C_2-C_8alkenyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkyl-C_1-C_8alkyl; C_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl which optionally may be substituted on the ring by hydroxy, C_1-C_8alkoxy, carboxy or C_2-C_8alkoxycarbonyl; thiazolyl; or pyridylalkyl; or R^1 and R^2 form together with the C-atoms to which they are attached a 5 to 10 membered aryl or heteroaryl residue, the latter comprising one or two heteroatoms selected from N, O and S; or

each of R^5 and R^6 independently is hydrogen; halogen; cyano; C_1-C_8alkyl; C_2-C_8alkenyl; C_2-C_8alkynyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkylC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl;

each of R^7, R^8 and R^9 is independently hydrogen; hydroxy; substituted or unsubstituted 5 or 6 membered heterocyclic ring comprising 1, 2 or 3 heteroatoms selected from N, O and S;

C_1-C_8alkyl; C_2-C_8alkenyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkylC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl; C_1-C_8alkoxy; -CF_3; -$CONR^{10}R^{11}$; -$N(R^{10})(R^{11})$; C_3-C_8cycloalkylC_1-C_8alkyl; heteroC_3-C_8cycloalkyl; carboxy; C_1-C_8alkoxycarbonyl; -$SO_2N(R^{10})R^{11}$; halogenoC_1-C_8alkyl; or R^7 and R^8 form together with the carbon atoms to which they are attached, a 5 or 6 membered heteroaryl radical comprising 1, 2 or 3 heteroatoms selected from N, O and S;

in free base or salt form.

By carbocyclic or heterocyclic residues are to be understood saturated or unsaturated cycloalkyl or heterocycloalkyl optionally condensed, e.g. to 1 or 2 benzene rings and/or to a further heterocyclic ring. Aryl means an aromatic carbocyclic residue. Heteroaryl is an aromatic heterocyclic system, e.g. a 5 or 6 membered aromatic heterocyclic residue optionally condensed, e.g. to 1 or 2 benzene rings and/or to a further heterocyclic ring. Examples of carbocyclic, heterocyclic, aryl or heteroaryl residues as mentioned above include C_6-C_{10}alkyl, C_1-C_9heteroalkyl, cyclopropyl, cyclohexyl, phenyl, naphthyl, 1,2,3,4-tetrahydronaphthalenyl, morpholinyl, piperazinyl, tetrazinyl, piperidyl, pyridyl, purinyl, pyrimidinyl, N-methyl-aza-cyclopentan-2-yl, N-methyl-aza-cycloheptan-4-yl, indolyl, quinolinyl, isoquinolinyl, 1,2,3,4-tetrahydroquinolinyl, benzothiazolyl, thiazolyl, imidazolyl, benzimidazolyl, benzoxadiazolyl, benzotriazolyl, indanyl, oxadiazolyl, pyrazolyl, triazolyl, and tetrazolyl.

Alkyl groups or alkyl moieties may be linear or branched. Alkyl, alkoxy, alkenyl, cycloalkyl, carbocyclic or heterocyclic residues, aryl or heteroaryl may be unsubstituted or substituted by one or more substituents selected from -OH; -COOH; -$C(O)NH_2$; nitro; halogen; cyano; -$C(NH_2)$=NOH; C_3-C_6-N-heteroaryl; C_3-C_5-N-heterocycloalkyl; C_1-C_8alkyl; C_1-C_8alkoxy; -$N(R^{10})R^{11}$; C_3-C_6cycloalkyl; phenyl; and morpholinyl.

Halogen may be F, Cl, Br, or I.

The compounds of the invention may exist in free form or in salt form, e.g. addition salts with e.g. organic or inorganic acids, for example trifluoroacetic acid or hydrochloride acid, or salts obtainable when they comprise a carboxy group, e.g. with a base, for example alkali salts such as sodium, potassium, or substituted or unsubstituted ammonium salts.

In formula I the following significances are preferred independently, collectively or in any combination or sub-combination:

(a) X is $=CR^0$;

(b) R^0 is hydrogen; halogen, e.g. Cl; C_1-C_8alkyl, e.g. methyl or ethyl; preferably hydrogen;

(c) R^1 is hydrogen; C_1-C_8alkyl, e.g. methyl or ethyl; morpholino; $-SO_2N(R^{10})R^{11}$; $-N(CH_3)C(O)CH_3$; hydroxyethyl; hydroxybutyl; 4-methyl-1-piperazinyl; C_1-C_8alkoxy, e.g. methoxy; C_5-C_{10}aryl, e.g. phenyl; or form together with R^2 and the C-atoms to which R^1 and R^2 are attached 5 to 10 membered aryl or heteroaryl, the latter comprising 1 or 2 nitrogen atoms; preferably hydrogen;

(d) R^2 is hydrogen; hydroxy; C_1-C_8alkyl, e.g. methyl or ethyl; carbamoyl; halogen, e.g. Cl, Br; $-SO_2N(R^{10})R^{11}$; hydroxyethyl; hydroxybutyl; 4-methyl-1-piperazinyl; methoxy; phenyl; or forms together with R^1 and the C-atoms to which R^1 and R^2 are attached 5 to 10 membered aryl or heteroaryl, the latter comprising 1 or 2 nitrogen atoms; preferably hydrogen, methyl, ethyl, butyl or phenyl;

(e) R^3 is hydrogen; halogen, e.g. Cl, Br; hydroxy; C_1-C_8alkyl, e.g. methyl or ethyl; methoxy; hydroxyethyl; hydroxybutyl; carboxy; carbamoyl; $-SO_2N(R^{10})R^{11}$; 4-methyl-1-piperazinyl; tetrazolyl; -C(O)NH(OH); or forms together with R^4 and the N and C atoms to which R^3 and R^4 are attached a 6 membered heterocyclic residue comprising at least one bond; preferably $-SO_2NH_2$;

(f) R^4 is hydrogen; or forms together with R^3 and the N and C atoms to which R^3 and R^4 are attached a 6 membered heterocyclic residue comprising at least one double bond; preferably hydrogen;

(g) R^5 is hydrogen; methyl; ethyl; Cl; Br; cyano; preferably hydrogen;

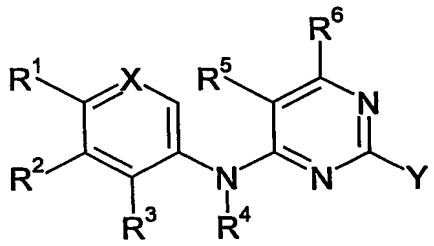
(h) R^6 is hydrogen;

(i) Each of R^7 and R^9 independently is hydrogen; hydroxy; methoxy; hydroxymethyl; 2-hydroxyethoxy; amino; dimethyl-amino; N-imidazolylethoxy; 2-methyl-N-imidazolylethoxy; $-NH-C(O)CH_3$; N-methyl-aza-cyclopentan-2-yl; N-methyl-aza-cycloheptan-4-yl; $NH-CH_2$-3-pyridyl; 1-piperidyl-ethoxy; 1,2,4-triazolyl-1-ethoxy; N-morpholino-ethoxy; $-SO_2N(R^{10})R^{11}$; CF_3 or R^7 forms together with R^8 and the C-atoms to which R^7 and R^8 are attached a 5 membered aryl or heteroaryl residue, e.g. bridged by -NH-CH=CH-, -CH=CH-NH-, -NH-N=CH-, -CH=N-NH-, -NH-N=N- or -N=N-NH-; preferably hydrogen; methoxy; N-imidazolylethoxy, 2-methyl-N-imidazolylethoxy or N-morpholino-ethoxy;

(k) R^8 is hydrogen; 4-piperidyl; hydroxy; methoxy; carboxy; $-NH-C(O)CH_3$; morpholino; 4-amino-N-piperidyl; or forms with R^7 or R^9 and the C-atoms to which R^7 and R^8 are attached a 5 membered aryl or heteroaryl residue, e.g. bridged by –NH-CH=CH-, -CH=CH-NH-, –NH-N=CH-, –CH=N-NH-, –NH-N=N- or –N=N-NH-; preferably hydrogen; methoxy or hydroxy;

(l) Each of R^{10} and R^{11}, independently, is hydrogen; methyl; butyl; 2-propenyl; 2-hydroxyethyl; 2-hydroxyethoxy; cyclopropyl; 2-thiazolyl; preferably hydrogen or methyl.

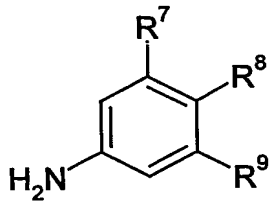
The present invention also provides a process for the production of a compound of formula I, comprising reacting a compound of formula II



(II)

wherein R^1, R^2, R^3, R^4, R^5, R^6 and X are as defined above, and Y is a leaving group, preferably halogen such as bromide, iodine, or in particular chloride;

with a compound of formula III



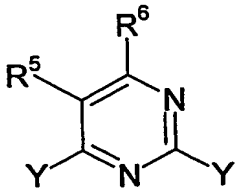
(III)

wherein R^7, R^8 and R^9 are as defined above;

and recovering the resulting compound of formula I in free or in form of a salt, and, where required, converting the compound of formula I obtained in free form into the desired salt form, or vice versa.

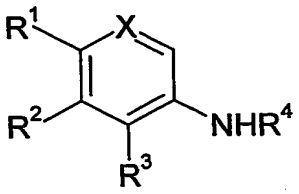
The process may be performed according to methods known in the art, e.g. as described in examples 1 to 4.

The compound of formula II used as starting materials may be obtained by reacting a compound of formula IV



(IV)

with a compound of formula V



(V)

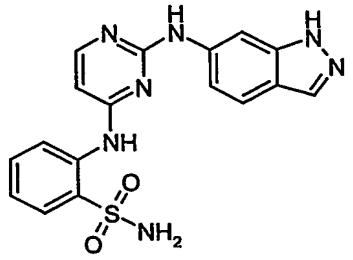
wherein R^1, R^2, R^3, R^4, R^5, R^6, Y and X are as defined above.

The compounds of formula IV and V are known or may be produced in accordance with known procedures.

The following abbreviations are employed: APC = allophycocyanine, BINAP = 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, cDNA = complementary DNA, DCM = dichloromethane, DIAD = diisopropyl azodicarboxylate, DMAP = 4-dimethylaminopyridine, DMF = dimethylformamide, DMSO = dimethylsulfoxide, DMF = dimethylformamide; Pmc = 2,2,5,7,8-pentamethylchroman; tBu = *tert.*-butyl; DIPCDI = N,N'-diisopropylcarbodiimid; DTT = 1,4-dithio-D,L-treitol, DNA = deoxyribonucleic acid, EDTA = ethylenediaminetetra-acetic acid, Lck = lymphoid T-cell protein tyrosine kinase, LAT-11 = linker for activation of T cell , RT = room temperature; RT-PCR = reverse transcription polymerase chain reaction, MS = molecular ion (e.g. $M+H^{1+}$) determined by electrospray mass spectroscopy; Eu = europium; ZAP-70 = zeta chain-associated protein of 70 kD; Syk = p72syk protein tyrosine kinase; SA = streptavidin.

The following examples illustrate the invention.

Example 1: 2-[2-(1H-Indazol-6-ylamino)-pyrimidin-4-ylamino]-benzenesulfonamide



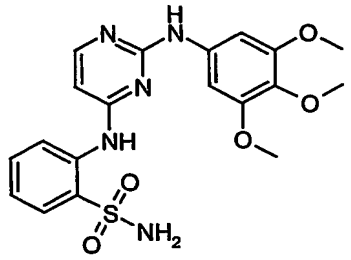
(a) 2-(2-Chloro-pyrimidin-4-ylamino)-benzenesulfonamide: To a suspension of 8.52 g (49.47 mmol) 2-aminobenzenesulfonamide in 200 ml isopropanol is added 22.1 g (148.42 mmol, 3 equivalent) 2,4-dichloropyrimidine and 20 ml 10 M hydrochloric acid (200 mmol, 4 equivalent). The suspension is stirred at 60°C for 2 h 15 min. The reaction mixture is dilluted with 2 l ethyl acetate and 500 ml water is added. The pH is adjusted to 8-9 by addition of sodium bicarbonate. The layers are separated and the aqueous layer is reextracted with 500 ml ethyl acetate. The organic layers are dried with sodium sulfate, filtered and evaporated to a volume of 300 ml. A crystalline precipitate is formed and removed by filtration (side product). The filtrate is evaporated to 100 ml whereupon the product crystallizes to give 2-(2-chloro-pyrimidin-4-ylamino)-benzenesulfonamide (97% purity by HPLC). The mother liquor of this cristallisation is further purified by column chromatography and crystallisation to give further 2-(2-chloro-pyrimidin-4-ylamino)-benzenesulfonamide.

(b) 2-[2-(1H-Indazol-6-ylamino)-pyrimidin-4-ylamino]-benzenesulfonamide: To a suspension of 7.25 g (25.46 mmol) 2-(2-Chloro-pyrimidin-4-ylamino)-benzenesulfonamide and 4.07 g (30.55 mmol, 1.2 equivalent) 6-aminoindazole in 400 ml isopropanol is added 13 ml conc. HCl* (130 mmol, 5 equivalent). The suspension is refluxed for 4 h 30 min. The reaction mixture is dilluted with 1.5 l ethyl acetate and 1 l water is added. The pH is adjusted to 8-9 by addition of sodium bicarbonate. The layers are separated and the aqueous layer is re-extracted with 500 ml ethyl acetate. The organic layers are dried with sodium sulfate, filtered and evaporated to a volume of 300 ml. A crystalline precipitate (1.01 g) is formed and removed by filtration (side product). The filtrate is purified by chromatography on 200 g silica gel eluting with ethyl acetate/methanol 95/5 v/v. Upon evaporation crystalls are formed which are filtered to give the title compound.

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.42 (s, 1H), 8.34 (d, 1h), 8.28 (d, 1H), 8.27 (s, 1H), 7.93 (s, 1H, 7.88 (d, 1H), 7.62 (m, 2H), 7.32 (d, 1H), 7.24 (t, 1H), 6.40 (d, 1H).

MS *m/z* (%): 382 (M+H, 100);

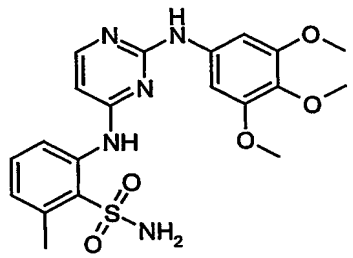
Example 2: 2-[2-(3,4,5-Trimethoxy-phenylamino)-pyrimidin-4-ylamino]-benzenesulfonamide



The title compound is prepared from 2-(2-chloro-pyrimidin-4-ylamino)-benzenesulfonamide as described in Example 1 using 3,4,5-Trimethoxy-phenylamine instead of 6-aminoindazole in step (b).

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.18 (s, 1H), 8.22 (d, 1H), 8.17 (d, 1H), 7.89 (d, 1H), 7.55 (t, 1H), 7.25 (t, 1H), 7.14 (s, 2H), 6.40 (d, 1H), 3.69 (s, 6H), 3.62 (s, 3H). MS *m/z* (%): 432 (M+H, 100);

Example 3: 2-methyl-6-[2-(3,4,5-Trimethoxy-phenylamino)-pyrimidin-4-ylamino]-benzenesulfonamide

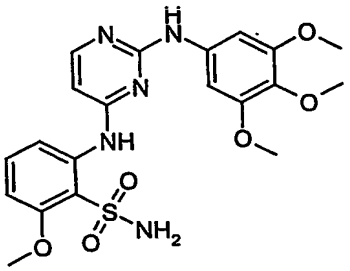


The tilte compound is prepared as described in Example 1 with the difference that in step (a) 2-amino-6-methyl-benzenesulfonamide is used instead of 2-aminobenzenesulfonamide.

2-Amino-6-methyl-benzenesulfonamide may be prepared as described by Girard, Y el al.; J. J. Chem. Soc. Perkin Trans. I ***1979****, 4, 1043-1047:* Under an atmosphere of nitrogen m-toluidin (32.1 g, 32.5 ml, 0.30 mmol) is added dropwise to a solution of chlorosulfonyl isocyanate (51.3 ml, 83.6 g, 0.59 mmol) in nitroethane (400 ml) at –55 – 49°C. The cold bath is removed and the mixture allowed to warm to –8°C, whereupon aluminium chloride (51 g, 0.38 mmol) is added. Heating the mixture to 100°C for 20 min forms a clear brown solution, which is cooled to RT and poured on ice. After filtration, washing with ice water and diethyl ether the precipitate is collected and dissolved in dioxane (300 ml). Water (1000 ml) and conc. HCl (1500 ml) are

added to form a suspension, which is heated to 120°C for 18h. After cooling to RT the clear brown solution is washed with diethyl ether/hexane (1400 ml, 1/1 v/v) and adjusted to pH = 8 by addition of sodium carbonate. Extraction using ethyl acetate (2 x 1000 ml), washing of the organic phase with water (500 ml) and brine (500 ml), drying (magnesium sulfate) and concentration yields a brown solid, which is purified by chromatography on silica using methylene chloride/ethanol (100/1 v/v) to yield the desired product as a white solid.
Melting point: 72-75°C (Propan-2-ol);
^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (s, 3H, Me), 3.63 (s, 3H, OMe), 3.68 (s, 6H, OMe), 6.31 (d, J = 5Hz, 1H, pyrimidine CH), 7.07 (d, J = 8Hz, 1H, arom. CH), 7.15 (s, 2H, arom. CH), 7.40 (t, J = 8Hz, 1H, arom. CH), 7.65 (s, 2H, SO_2NH_2), 8.04 (d, J = 8Hz, 1H, arom. CH), 8.12 (d, J = 5Hz, 1H, pyrimidine CH), 9.14 (s, 1H, NH), 9.40 (s, 1H, NH).
MS (ES$^+$) *m/z*: 446 (MH^+), 468 (MNa^+)
MS (ES$^-$): 444 $(M-H)^-$

Example 4: 2-Methoxy-6-[2-(3,4,5-trimethoxy-phenylamino)-pyrimidin-4-ylamino]-benzenesulfonamide



The title compound is prepared as described in Example 1 with the difference that in step (a) 2-amino-6-methoxy-benzenesulfonamide is used instead of 2-Amino-6-methyl-benzenesulfonamide.
2-Amino-6-methoxy-benzenesulfonamide may be prepared from 12.3 g of *meta*-anisidine following an analogous procedure as described in Example 1a. NMR (400 MHz, DMSO-d_6): δ3.62 (s, 3H, OMe), 3.69 (s, 6H, OMe), 3.91 (s, 3H, OMe), 6.31 (d, J = 5Hz, 1H, pyrimidine CH), 6.86 (d, J = 8Hz, 1H, arom. CH), 7.12 (s, 2H, arom. CH), 7.43 (t, J = 8Hz, 1H, arom. CH), 8.01 (d, J = 8Hz, 1H, arom. CH), 8.11 (d, J = 5Hz, 1H, pyrimidine CH), 9.18 (s, 1H, NH), 9.79 (br, 1H, NH).
MS (ES$^+$): 462.2 (MH^+), 484.2 (MNa^+)
MS (ES$^-$): 460.3 $(M-H)^-$

In the following examples compounds of formula I wherein X is $=CR^0-$ and R^6 is H are prepared according to the invention

Ex = Example										MS data		
EX	**R^0**	**R^1**	**R^2**	**R^3**	**R^4**	**R^5**	**R^7**	**R^8**	**R^9**	*ES+	*ES-	*EI
5	-H	-H	-H	-OH	-H	-H	-O-(1-methyl)-azacyclohept-4-yl	-H	-H	406	404	
6	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-(1-methyl)-azacyclohept-4-yl	-H	-H	469.3		
7	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-2-(1-methyl-azacyclopent-2-yl)-ethyl	-H	-H	469.3		
8	-H	-H	-H	-OH	-H	-H	-O-2-(1-piperidyl)-ethyl	$-OCH_3$	-H	436.3	434.4	
9	-H	-H	-H	-OH	-H	-H	-O-2-(1-methyl-azacyclopent-2-yl)-ethyl	-H	-H	406	404	
10	-H	-H	-H	-COOH	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	397	395	
11	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-O-CH_2CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H	496	494	
12	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-2-(1-piperidyl)-ethyl	$-OCH_3$	-H	499.2	497.3	
13	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	-OH	$-OCH_3$			
14	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-O-CH_2CH_2$-1-methyl-imidazol-1-yl	-H	-H	466	464	
15	-H	-H	-H	-OH	-H	-H	-O-2-[1-(1,2,4-triazolyl)]-ethyl	-H	-H	390	388	
16	-H	-H	-H	-OH	-H	-H	-O-2-hydroxyethyl	$-OCH_3$	-H	369.4	367.3	
17	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-2-hydroxyethyl	$-OCH_3$	-H			431
18	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H			
19	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-2-[1-(1,2,4-triazolyl)]-ethyl	-H	-H			452
20	-H	-H	-H	$-SO_2NHCH_3$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
21	-H	-H	-H	$-SO_2NH_2$	-H	-H	-NH-N=N-		-H		381	
22	-H	-H	-H	$-SO_2NHCH_3$	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H	496	494	
23	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-2-(1-piperidyl)-ethyl	-H	-H	469	467	
24	-H	-H	-H	-5-(1,2,3,4-tetrazolyl)	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	421		
25	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	452	450	
26	-H	-H	-H	$-SO_2NH$-cyclopropyl	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	472.2	470.3	
27	-H	-H	-H	-OH	-H	-H	-O-2-(1-piperidyl)-ethyl	-H	-H	406		

28	-H	-H	-H	-COOH	-H	-H	-4-morpholino	-H	-H			
29	-H	-H	-H	-OH	-H	-H	-O-$CH_2CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H	433	431	
30	-H	-H	-H	-C(O)NHOH	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	412	410	
31	-H	-H	-H	$-SO_2NHCH_3$	-H	-H	-CH=N-NH-		-H	396	394	
32	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-2-(4-morpholino)ethyl	-H	-H	471	469	
33	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	$-OCH_3$	-H	402	400	
34	-H	-H	-H	$-SO_2NH-CH_2CH_2-OH$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	476	474	
35	-H	-H	-H	$-SO_2N(CH_3)_2$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	460.3	458.3	
36	-H	-H	-H	-OH	-H	-H	-O-2-(4-morpholino)ethyl	-H	-H	408	406	
37	-H	-H	-H	-H	-H	-H	$-OCH_3$	-OCH3	$-OCH_3$	353	351	
38	-H	-H	-H	$-SO_2NH_2$	-H	-H	-CH=N-NH-		-H			381
39	-H	-H	-H	-OH	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	369	367	
40	-H	-H	-H	$-SO_2NHCH_3$	-H	-H	-O-CH_2CH_2-1-imidazolyl	-H	-H			
41	-H	-H	-H	-COOH	-H	-H	amino	-H	-H	322		
42	-H	-H	-H	$-SO_2NH_2$	-H	-H	-O-$CH_2CH_2CH_2$-1-imidazolyl	-H	-H	466.2	464.3	
43	-H	-H	-H	-COOH	-H	-H	$-N(CH_3)_2$	-H	-H			
44	-H	-H	-H	-5-(1,2,3,4-tetrazolyl)	-H	-H	-NH-C(O)CH_3	-H	-H	388	386	
45	-H	-H	-H	$-SO_2NHCH_3$	-H	-H	-NH-N=CH-		-H			
46	-H	-H	-H	-COOH	-H	-H	-OH	-H	-H			
47	-H	-H	-H	$-SO_2NH-CH_2CH_2CH_3$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	474	472	
48	-H	-H	-H	-COOH	-H	-H	-H	-4-piperidyl	-H			
49	-H	-H	-H	-COOH	-H	-H	$-CH_2$-OH	-H	-H			
50	-H	-H	-H	-OH	-H	-H	-O-CH_2CH_2-1-imidazolyl	-OCH3	-H			
51	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	-O-CH_2CH_2-1-imidazolyl	-H	-H	466		
52	-H	-H	-H	$-SO_2NH-CH_2CH_2-OH$	-H	-H	-O-CH_2CH_2-1-imidazolyl	-H	-H	496	494	
53	-H	-H	-H	$-CH_2OH$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
54	-H	-H	-H	$-C(O)NH_2$	-H	-H	amino	-H	-H	321		
55	-H	-H	-H	$-SO_2NH_2$	-H	-H	-CH=CH-NH-		-H	381		
56	-H	-H	-H	-5-(1,2,3,4-tetrazolyl)	-H	-H	$-NHCH_2$-3-pyridyl	-H	-H		435	

57	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	-H	$-OCH_3$	402		
58	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	-CH=N-NH-		-H	396		
59	-H	-H	-H	$-SO_2NH_2$	-H	-H	-NH-CH=CH-		-H		379	
60	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_2CH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	460	458	
61	-H	-H	-H	-COOH	-H	-H	-H	-4-morpholino	-H			
62	-H	-H	-H	-COOH	-H	-H	-H	-1-(4-amino)-piperidyl	-H			
63	-H	-H	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	-H	-H	372	370	
64	-H	-H	-H	$-SO_2N(CH_3)_2$	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	480	478	
65	-H	-H	-H	$-SO_2NH_2$	-H	-Br	-NH-N=CH-		-H	461		
66	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	-O-CH2CH2-1-imidazolyl	$-OCH_3$	-H	496		
67	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	446		
68	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	-N=N-NH-		-H	397.2	395.2	
69	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	$-O-CH_2CH_2$-1-methyl-imidazol-1-yl	-H	-H	480		
70	-H	-H	-H	$-SO_2NH_2$	-H	-Br	-CH=N-NH-		-H	461.3	458.1/460	
71	-H	-H	-H	$-SO_2NH_2$	-H	$-CH_3$	-NH-N=CH-		-H	396		
72	-H	4-morpholino	-H	-H	-H	-H	$-OCH_3$	-H	$-OCH_3$			
73	-H	$-SO_2NH-CH_2CH_2-O-CH_2CH_2-OH$	-H	-H	-H	-H	-H	$-N(CH_3)-C(O)CH_3$	-H			
74	-H	$-SO_2NH_2$	-H	-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
75	-H	$-SO_2NH_2$	-H	-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H			
76	-H	$-SO_2NH-CH_2CH_2-O-CH_2CH_2-OH$	-H	-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	520	518	

77	-H	-N(CH_3) C(O)CH_3	-H	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3	424	422	
78	-H	-CH_2CH_2-OH	-H	-H	-H	-H	-SO_2NH-$CH_2CH_2CH_2CH_3$	-H	-H			
79	-H	-SO_2NH_2	-H	-H	-H	-H	-OCH_3	-H	-OCH_3			
80	-H	-CH_2CH_2-OH	-H	-H	-H	-CH_3	-OCH_3	-OCH_3	-OCH_3	411	409	
81	-H	-SO_2NH_2	-H	-H	-H	-H	-O-CH_2CH_2-1-imidazolyl	-H	-H			
82	-H	-CH_2CH_2-OH	-H	-H	-H	-H	-O-CH_2CH_2-1-imidazolyl	-H	-H			
83	-H	4-morpholino	-H	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3	438		
84	-H	-CH_2CH_2-OH	-H	-H	-H	-H	-OCH_3	-H	-OCH_3			
85	-H	-SO_2NH_2	-H	-H	-H	-CH_3	-O-CH_2CH_2-1-imidazolyl	-OCH_3	-H	496.3	494.3	
86	-H	-SO_2NH_2	-H	-H	-H	-H	-OH	-H	-H			
87	-H	-O-CH_2CH_2-OH	-H	-H	-H	-H	-O-CH_2CH_2-1-imidazolyl	-H	-H			
88	-H	-SO_2NH-2-thiazolyl	-H	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3	515	513	
89	-H	-4-methyl-piperazinyl	-H	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3	451	449	
90	-H	-O-CH_2CH_2-CH2-OH	-H	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3			
91	-H	-H	-SO_2NH-2-propenyl	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3	472	470	
92	-H	-H	-SO_2NH_2	-H	-H	-H	-OCH_3	-OCH_3	-OCH_3			
93	-H	-H	-OH	-H	-H	-H	-O-(1-methyl)-azacyclohept-4-yl	-H	-H	406.3	404.3	
94	-H	-H	-OH	-H	-H	-H	-O-CH_2CH_2-OH	-OCH_3	-H	369	367	
95	-H	-H	-SO_2NH_2	-H	-H	-Br	-OCH3	-OCH_3	-OCH_3	510.1/ 512.1	508.1/ 510.2	
96	-H	-H	-SO_2NH_2	-H	-H	-H	-CH=N-NH-		-H	382		
97	-H	-H	-SO_2NH_2	-H	-H	-CH_3	-OCH_3	-OCH_3	-OCH_3	446	444	
98	-H	-H	-SO_2NH_2	-H	-H	-H	-O-CH_2CH_2-1-imidazolyl	-OCH_3	-H	482	480	
99	-H	-H	-OH	-H	-H	-H	-O-CH_2CH_2-1-piperidyl	-OCH_3	-H	436.3	434.3	
100	-H	-H	-OH	-H	-H	-H	-O-CH_2CH_2-1-imidazolyl	-OCH_3	-H	419	417	
101	-H	-H	-SO_2NH_2	-H	-H	-H	-O-CH_2CH_2-1-imidazolyl	-H	-H	452	450	

102	-H	-H	$-CH_3$	-H	-H	-C≡N	$-OCH_3$	$-OCH_3$	$-OCH_3$	392		
103	-H	-H	$-SO_2NH_2$	-H	-H	-H	-NH-N=CH-		-H	382		
104	-H	-H	-OH	-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	369	367	
105	-H	-H	$-SO_2NHCH_3$	-H	-H	$-CH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	460	458	
106	-H	-H	-OH	-H	-H	-H	-OH	-COOH	$-OCH_3$			
107	-H	-H	-OH	-H	-H	-H	$-O-CH_2CH_2$-1-piperidyl	-H	-H	406	404	
108	-H	-H	$-SO_2NH$-2-propenyl	-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	492.3	490.3	
109	-H	-H	$-SO_2NH_2$	-H	-H	-Br	$-O-CH_2CH_2$-1-(1-methyl)-imidazolyl	-H	-H	544.1/546	542/544.2	
110	-H	-H	$-SO_2NH_2$	-H	-H	-H	$-O-CH_2CH_2$-OH	$-OCH_3$	-H			
111	-H	-H	-OH	-H	-H	-H	-O-(1-methyl)-azacyclopent-2-yl	-H	-H			
112	-H	-H	-OH	-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	389	387	
113	-H	-H	-OH	-H	-H	-H	$-O-CH_2CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H	433.4	431.4	
114	-H	-H	$-SO_2NH_2$	-H	-H	-H	$-OCH_3$	-H	$-OCH_3$			
115	-H	-H	-OH	-H	-H	-H	$-OCH_3$	$-OCH_3$	-H	339	337	
116	-H	-H	$-SO_2NHCH_2-CH_2CH_2CH_3$	-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	488	486	
117	-H	-H	$-SO_2NH-CH_3$	-H	-H	$-CH_3$	$-O-CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H	510	508	
118	-H	-H	$-SO_2NHCH_2-CH_2CH_2CH_3$	-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	08	506	
119	-H	-H	-OH	-H	-H	-H	$-O-CH_2CH_2$-4-morpholino	-H	-H	408		
120	-H	-H	-OH	-H	-H	-H	-NH-N=CH-		-H	319	317	
121	-H	-H	-OH	-H	-H	-H	-CHN-NH-		-H	319	317	
122	-H	-H	-OH	-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H			
123	-H	-H	$-SO_2NH-CH_3$	-H	-H	$-CH_2-CH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	474.3	472.3	
124	-H	-H	$-SO_2NH_2$	-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
125	-Cl	-H	-H	-OH	-H	-H	-O-CH2CH2-1-piperidyl	$-OCH_3$	-H	470.3	468.3	
126	-H	-CH=N-NH-		-H	-H	-H	$-OCH_3$	-H	$-OCH_3$	363	361	
127	-H	$-OCH_3$	-OH	-H	-H	-H	-OH	$-OCH_3$	-H			
128	-Cl	-H	-H	-OH	-H	-H	-O-(1-methyl)-	-H	-H	440		

							azacyclohept-4-yl					
129	-H	-H	$-CH_3$	$-SO_2NH_2$	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	466	464	
130	-Cl	-H	-H	-OH	-H	-H	-O-(1-methyl)-azacyclopent-2-yl	-H	-H	440	438	
131	-H	$-OCH_3$	-OH	-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
132	-H	nitro	-H	-OH	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	414	412	
133	-H	-N=CH-CH=CH-		-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
134	-H	-CH=N-NH-		-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	393	391	
135	-H	$-OCH_3$	-OH	-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H			
136	-H	$-OCH_3$	-OH	-H	-H	-H	$-OCH_3$	-H	$-OCH_3$			
137	-Cl	-H	-H	-OH	-H	-H	$-O-CH_2CH_2-CH_2$-1-imidazolyl	$-OCH_3$	-H	467	465	
138	-Cl	-H	-H	-OH	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$			
139	-H	$-CH_3$	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	446		
140	-H	$-OCH_3$	-OH	-H	-H	-H	$-O-CH_2CH_2-OH$	$-OCH_3$	-H	399	397	
141	-H	$-OCH_3$	-OH	-H	-H	-H	-O-(1-methyl)-azacyclohept-4-yl	-H	-H	436		
142	-H	$-CH_3$	-H	$-SO_2NH_2$	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	-H	-H	466	464	
143	-Cl	-H	-H	-OH	-H	-H	$-O-CH_2CH_2$-1-(1,2,4-triazolyl)	-H	-H	424	422	
144	-H	$-OCH_3$	-OH	-H	-H	-H	$-O-CH_2CH_2$-(1-methyl)-azacyclopent-2-yl	-H	-H	436	434	
145	-Cl	-H	-H	-OH	-H	-H	$-O-CH_2CH_2$-1-piperidyl	-H	-H			
146	-H	$-OCH_3$	-OH	-H	-H	-H	tri-fluoro-methyl	-H	-H			
147	-H	-N=CH-CH=CH-		-H	-H	-H	$-O-CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H			
148	-H	$-OCH_3$	-OH	-H	-H	-H	$-O-CH_2CH_2CH_2$-1-imidazolyl	$-OCH_3$	-H	463	461	
149	-H	$-OCH_3$	-OH	-H	-H	-H	$-O-CH_2CH_2$-1-piperidyl	$-OCH_3$	-H	466.4	464.4	
150	-H	-NH-N=CH-		-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	393		
151	-H	-H	-OH	$-CH_2CH_2CH_2$-		-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	409	407	
152	$-CH_3$	-H	$-CH_3$	-OH	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	397		
153	-H	phenyl	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	508	506	
154	$-CH_3$	-H	-H	$-SO_2NH_2$	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	446	444	
155	-H	-CH=N-NH-		-H	-H	-H	-NH-N=CH-		-H			
156	-Cl	-H	-H	-OH	-H	-H	$-O-CH_2CH_2-OH$	$-OCH_3$	-H			
157	-H	-CH=N-NH-		-H	-H	-H	-CH-N=NH-					

158	-Cl	-H	-H	-OH	-H	-H	-O-CH_2CH_2-4-morpholino	-H	-H	442	440	
159	-H	-OCH3	-OH	-H	-H	-H	-O-CH_2CH_2-1-piperidyl	-H	-H	436	434	
160	-Cl	-H	-H	-OH	-H	-H	-O-$CH_2CH_2CH_2$-1-imidazolyl	-H	-H			

In the following examples compounds of formula I wherein X is $=CR^0-$ and R^6 is H are prepared according to the invention

EX	R^1	R^2	R^3	R^4	R^5	R^7	R^8	R^9			
161	-CH=CH-CH=CH-		-H	-H	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	404		
162	-CH=CH-CH=CH-		-H	-H	-H	$-OCH_3$	-OH	$-OCH_3$	390	388	

ES+ means electrospray MS positive mode ; ES- means electrospray MS negative mode; and EL means electron impact MS.

The compounds of formula I and their pharmaceutically acceptable salts, hereinafter referred to as agents of the invention, exhibit valuable pharmacological properties when tested in vitro in cell-free kinase assays and in cellular assays, and are therefore useful as pharmaceuticals.

In particular the agents of the invention exhibit ZAP-70 and/or Syk protein tyrosine kinases inhibiting activity. More particularly the agents of the invention are active at the human ZAP-70 and/or Syk protein tyrosine kinases. ZAP-70 and/or Syk protein tyrosine kinase interaction of the agents of the invention may be demonstrated by their ability to prevent phosphorylation of e.g. LAT-11 (SEQ ID NO: 1) by human ZAP-70 protein tyrosine kinase and/or to prevent phosphorylation of e.g. polymeric glutamic acid-tyrosine (Glu, Tyr) by human Syk protein tyrosine kinase in, e.g. aqueous solution, e.g. as demonstrated in accordance with the following test methods.

Test I: Cell-free kinase assays: ZAP-70 and Syk Kinase assays

Cloning, expression and purification of recombinant ZAP-70 and/or Syk kinase: A nucleic acid encoding full-length human ZAP-70 (GenBank #L05148) is amplified from a Jurkat cDNA library by RT-PCR and cloned into the pBluescript KS vector (Stratagene, California, U.S.). The authenticity of the ZAP-70 cDNA insert is validated by complete sequence analysis. This donor plasmid is then used to construct a recombinant baculovirus transfer vector based on the plasmid pVL1392 (Pharmingen, California, U.S.) featuring in addition an N-terminal hexahistidine tag. Following co-transfection with AcNPV viral DNA, ten independent viral isolates are derived via plaque-purification, amplified on small scale and subsequently analyzed for recombinant ZAP-70 expression by Western Blot using a commercially available anti-ZAP-70 antibody (Clone 2F3.1, Upstate Biotechnology, Lake Placid, NY). Upon further amplification of one positive recombinant plaque, titrated virus stocks are prepared and used for infection of Sf9 cells grown in serum-free SF900 II medium (Life Technologies, Basel) under defined, optimized conditions.
ZAP-70 protein is isolated from the lysate of infected Sf9 cells by affinity chromatography on a Ni-NTAcolumn (Qiagen, Basel).
Recombinant full-length human Syk (GenBank #Z29630) are produced by analogous methods.

Preparation of LAT-11 (SEQ ID NO:1): The peptide used as a substrate in the ZAP-70 kinase assay is prepared in analogy with known methods, e.g. as known in the art of peptide synthesis

or as described in the following example. Where desired protecting groups known to the skilled artisan may temporarily be attached to one or more of the functional groups present. Protecting groups also include a polymer resin having suitable functional groups. Following a typical protocol compounds of the invention may for example be synthesized in a stepwise manner on a resin support, e.g. a poly-styrene based resin support. The α-amino group may, e.g., be protected by 9-fluorenyl-methoxycarbonyl (Fmoc) and the side-chain functional groups may, e.g., be protected by tert.butyl or triphenylmethane (Trt). The stepwise solid phase synthesis usually consists of repetitive cycles of α-amino group deprotection, washing, coupling (i.e., attachment of next amino acid residue to the growing peptide chain) and washing. After complete assembly of the peptide chain the terminal protecting group may be removed and, optionally, a labelling group may be coupled to the terminal amino group. The peptide may be cleaved from the resin support, side-chain protecting groups may be removed and the product may be purified following established methods of peptide chemistry.
The N-α Fmoc group of Fmoc-Asn(Trt)-oxymethyl-4-phenoxymethyl-co(polystyrene-1%-divinyl-benzene), content of Asn approx. 0.5 mmol/g, is cleaved using piperidine, 20% in DMF. Four equivalents per amino-group of Fmoc-amino acid protected in their side chains [Asp(OtBu), Glu(OtBu), Asn(Trt), Gln(Trt) and Tyr(tBu)] are coupled using DIPCDI and HOBt in DMF. After complete assembly of the peptide chain the terminal Fmoc-protecting group is removed with piperidine in DMF as before. L(+)-biotinyl-aminohexanoic acid is then coupled to the terminal amino group using DIPCDI and HOBt in DMF using four equivalents of the reagents for four days at RT. The peptide is cleaved from the resin support and all side-chain protecting groups are simultaneously removed by using a reagent consisting of 5% dodecylmethylsulfide and 5% water in TFA for two hours at RT. Resin particles are filtered off, washed with TFA and the product is precipitated from the combined filtrates by the addition of 10 to 20 volumes of diethyl ether, washed with ether and dried. The product is purified by chromatography on a C-18 wide-pore silica column using a gradient of acetonitrile in 2% aqueous phosphoric acid. Fractions containing the pure compound are collected, filtered through an anion-exchange resin (Biorad, AG4-X4 acetate form) and lyophilized to give the title compound. MS: 1958.0 $(M-H)^{-1}$

ZAP-70 Kinase assay: The activities of the agents of invention are determined in a homogenous ZAP-70 kinase assay based on time-resolved fluorescence resonance energy transfer. Briefly, 80 nM ZAP-70 are incubated with 80 nM Lck and 4 μM ATP in ZAP-70 kinase buffer (20 mM Tris, pH 7.5, 10 μM Na_3VO_4, 1 mM DTT, 1 mM $MnCl_2$, 0.01 % bovine serum albumin, 0.05 % Tween 20) for 1 hour at room temperature in a siliconized polypropylene tube. Then, the

selective Lck inhibitor PP2 (4-amino-5-(4-chloro-phenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine; Alexis Biochemicals) is added (final concentration 1.2 μM) and incubated for further 10 min. Ten μl of this solution is mixed with the 10 μl biotinylated peptide LAT-11 (1 μM) as substrate and 20 μl of serial dilutions of inhibitors and incubated for 4 hours at room temperature. The kinase reaction is terminated with 10 μl of a 10 mM EDTA solution in detection buffer (20 mM Tris, pH 7.5, 0.01 % bovine serum albumin, 0.05 % Tween 20). The detection phase is performed by addition of 50 μl europium (Eu)-labelled anti-phosphotyrosine antibody (e.g. Eu-PT66; final concentration 0.125 nM; Advant/Wallac) and 50 μl streptavidin-allophycocyanine (SA-APC; final concentration 40 nM) in detection buffer. After 1 hour incubation at room temperature fluorescence is measured, e.g., on the Victor2 Multilabel Counter (Wallac) at 665 nm. Background values (low control) are obtained in the absence of test samples and ATP and are subtracted from all values. Signals obtained in the absence of test samples are taken as 100% (high control). The inhibition obtained in the presence of test compounds was calculated as percent inhibition of the high control. The concentration of test compounds resulting in 50% inhibition (IC_{50}) was determined from the dose-response curves. In this assay, the agents of the invention have IC_{50} values in the range of 10 nM to 2 μM, preferably from 10 nM to 100 nM. Compound of Example 4 shows an IC_{50} value of 12 nM.

Syk Kinase assay: The activities of the agents of invention are determined in a heterogenous Syk kinase assay based on the dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) technology. This method utilizes europium chelate-labelled anti-phosphotyrosine antibodies to detect phosphate transfer by Syk to a polymeric glutamic acid-tyrosine (Glu, Tyr) substrate coated onto microtiter plates as described (Braunwalder AF, Yarwood DR, Sills MA, Lipson KE. Measurement of the protein tyrosine kinase activity of c-src using time-resolved fluorometry of europium chelates. Anal.Biochem. 1996;238(2):159-64). The amount of phosphorylation is then quantified with time-resolved, dissociation-enhanced fluorescence. Briefly, hundred μl of poly (Glu, Tyr) (4:1; 2 μg/ml in phosphate-buffered saline, PBS) are coated to ELISA plates overnight at room temperature. The poly (Glu, Tyr) solution is removed and 250 μl of 1% bovine serum albumin in PBS are added for one hour at room temperature. Plates are then washed three times with 350 μl of washing buffer (25 mM Tris-HCl, pH 7.4 containing 0.03% Tween-20). The kinase reaction is performed for one hour at room temperature by mixing serial dilutions of inhibitors in 30 μl with 30 μl of Syk kinase (20 ng/ml) and ATP (1 μM) in kinase buffer (20 mM Tris, pH 7.5, 10 μM Na_3VO_4, 1 mM DTT, 10 mM $MnCl_2$, 2 mM $MgCl_2$, 0.01 % bovine serum albumin, 0.05 % Tween 20). After washing the plates four times as described above 60 μl DELFIA europium N1-labelled anti-phosphotyrosine antibody PY20 (Advant/Wallac) are added (100 ng/ml in 50 mM

Tris-HCl, pH7.4, 150 mM NaCl, 20 µM Titriplex V, 0.2% bovine serum albumine, 0.05% Tween-20) and incubated for one hour at room temperature. Plates are washed eight times and 60 µl enhancement solution (Wallac) are added. Fluorescence is determined at 615 nm (Victor2; Wallac). High control values (100% signal) are obtained in absence of test samples and low control values (background) in absence of test samples and ATP. Low controls were subtracted from all values. The inhibition obtained in the presence of test compounds was calculated as percent inhibition of the high control. The concentration of test compounds resulting in 50% inhibition (IC_{50}) was determined from the dose-response curves. In this assay, the agents of the invention have IC_{50} values in the range of 100 nM to 10 µM, preferably from 100 to 1 µM. Compound of Example 129 has an IC_{50} value of 150 nM.

Test II: Cellular assay: Allogeneic Mixed Lymphocyte Reaction (MLR)

Agents of the invention exhibit T cell inhibiting activity. More particular the agents of the invention prevent T cell activation and/or proliferation in e.g. aqueous solution, e.g. as demonstrated in accordance with the following test method. The two-way MLR is performed according to standard procedures (J. Immunol. Methods, 1973, 2, 279 and Meo T. et al., Immunological Methods, New York, Academic Press, 1979, 227-39). Briefly, spleen cells from CBA and BALB/c mice (1.6×10^5 cells from each strain per well in flat bottom tissue culture microtiter plates, 3.2×10^5 in total) are incubated in RPMI medium containing 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco BRL, Basel, Switzerland), 50 µM 2-mercaptoethanol (Fluka, Buchs, Switzerland) and serially diluted compounds. Seven three-fold dilution steps in duplicates per test compound are performed. After four days of incubation 1 µCi ^{3}H-thymidine is added. Cells are harvested after an additional five-hour incubation period, and incorporated ^{3}H-thymidine is determined according to standard procedures. Background values (low control) of the MLR are the proliferation of BALB/c cells alone. Low controls are subtracted from all values. High controls without any sample are taken as 100% proliferation. Percent inhibition by the samples is calculated, and the concentrations required for 50% inhibition (IC_{50} values) are determined. In this assay, the agents of the invention have IC_{50} values in the range of 10 nM to 10 µM, preferably from 10 nM to 100 nM. Compound of Example 66 shows an IC_{50} value of 13 nM.

The agents of the invention are thus indicated for the prevention or treatment of disorders or diseases where ZAP-70 inhibition and/or Syk inhibition play a role. Therefore, the agent of the invention are, for example, useful to prevent and/or treat a vertebrate and more particularly a mammal, affected by immune system disorders, diseases or disorders mediated by T

lymphocytes, B lymphocytes, mast cells and/or eosinophils e.g. acute or chronic rejection of organ or tissue allo- or xenografts, atheriosclerosis, vascular occlusion due to vacular injury such as angioplasty, restenosis, hypertension, heart failure, chronic obstructive pulmonary disease, CNS disease such as Alzheimer disease or amyotrophic lateral sclerosis, cancer, infectious disease such as AIDS, septic shock or adult respiratory distress syndrome, ischemia/reperfusion injury e.g. myocardial infarction, stroke, gut ischemia, renal ailure or hermorrhage shock, or traumatic shock. The agent of the invention are also useful in the treatment and/or prevention of acute or chronic inflammatory diseases or disorders or autoimmune diseases e.g. rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, Hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes (type I and II) and the disorders associated with therewith, respiratory diseases such as asthma or inflammatory liver injury, inflammatory glomerular injury, cutaneous manifestations of immunologically-mediated disorders or illnesses, inflammatory and hyperproliferative skin diseases (such as psoriasis, atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis), inflammatory eye diseases, e.g. Sjoegren's syndrome, keratoconjunctivitis or uveitis, inflammatory bowel disease, Crohn's disease or ulcerative colitis. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.1 to about 100 mg/kg body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 2000 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

The agent of the invention may be administered by any conventional route, in particular parenterally, for example in the form of injectable solutions or suspensions, enterally, preferably orally, for example in the form of tablets or capsules, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising an agent of the invention in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms for oral administration contain, for example, from about 0.1 mg to about 500 mg of active substance. Topical administration is e.g. to the skin. A further form of topical administration is to the eye.

The agents of the invention may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents. For example, the agents of the invention may be used in accordance with the invention in combination with pharmaceutical compositions effective in various diseases as described above, e.g. with cyclosporins, rapamycins or ascomycins, or their immunosuppressive analogs, e.g. cyclosporin A, cyclosporin G, FK-506, sirolimus, everolimus; corticosteroids e.g. prednisone; cyclophosphamide; azathioprene; methotrexate; gold salts, sulfasalazine, antimalarials, brequinar; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine; other immuno-suppressive monoclonal antibodies, e.g. monoclonal antibodies to leukocyte receptors, e.g. MHC, CD2, CD3, CD4, CD7, CD25, CD28, CD40, CD45, CD58, CD80, CD86, CD152, CD137, CD154, ICOS, LFA-1, VLA-4 or their ligands; or other immunomodulatory compounds, e.g. CTLA4Ig.

In accordance with the foregoing, the present invention also provides:

(1) An agent of the invention for use as a pharmaceutical;

(2) An agent of the invention for use as a ZAP-70 and/or Syk tyrosine kinase inhibitor, for example for use in any of the particular indications hereinbefore set forth;

(3) A pharmaceutical composition, e.g. for use in any of the indications herein before set forth, comprising an agent of the invention as active ingredient together with one or more pharmaceutically acceptable diluents or carriers therefor.

(4) A method for the treatment of any of particular indication hereinbefore set forth in a subject in need thereof which comprises administering an effective amount of an agent of the invention;

(5) The use of an agent of the invention for the manufacture of a medicament for the treatment or prevention of a disease or condition in which ZAP-70 and/or Syk tyrosine kinase activation plays a role or is implicated;

(6) A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a ZAP-70 and/or Syk tyrosine kinase inhibitor, e.g. an agent of the invention and a second drug substance, said second drug substance being for example for use in any of the particular indications hereinbefore set forth.

(7) A combination comprising a therapeutically effective amount of a ZAP-70 and/or Syk tyrosine kinase inhibitor, e.g. an agent of the invention, and a second drug substance, said second drug substance being for example as disclosed above.

Preferred compounds of formula I for use in accordance with the invention are compounds of Examples 1 to 4.

SEQUENCE LISTING

<110> Novartis AG

<120> Organic Compound

<130> 4-32366P1

<160> 1

<170> PatentIn version 3.1

<210> 1

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> LAT-11 is an artificial sequence for use in the ZAP-70 kinase ass

ay

<220>

<221> MISC_FEATURE

<222> (1)..(15)

<223>

<220>

<221> MISC_FEATURE

<222> (1)..(15)

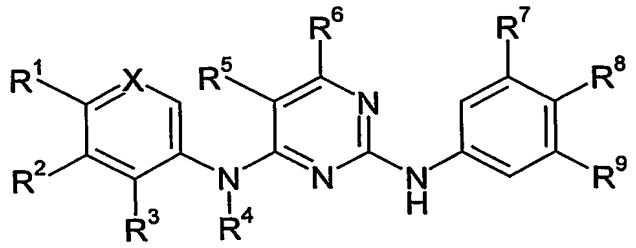
<223> X =L(+)-biotinyl-aminohexanoyl

<400> 1

Xaa Glu Glu Gly Ala Pro Asp Tyr Glu Asn Leu Gln Glu Leu Asn
1 5 10 15

Claims:

1. A compound of formula I



(I)

wherein

X is $=CR^0-$ or =N-;

each of R^0, R^1, R^2, R^3 and R^4 independently is hydrogen; hydroxy; C_1-C_8alkyl; C_2-C_8alkenyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkyl-C_1-C_8alkyl; C_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl which optionally may be substituted on the ring by hydroxy, C_1-C_8alkoxy, carboxy or C_1-C_8alkoxycarbonyl; or residue R^3 and R^4 form together with the nitrogen and carbon atoms to which they are attached a carbocyclic ring having 5 to 10 ring atoms or a heterocyclic ring having 5 to 10 ring atoms and comprising 1, 2 or 3 heteroatoms selected from N, O and S; or each of R^1, R^2 and R^3, independently, is halogen; halogenoC_1-C_8alkyl; hydroxy; C_1-C_8alkoxy; hydroxyC_1-C_8alkoxy; C_5-C_{10}aryl; carboxy; C_2-C_8alkoxycarbonyl; C_5-C_{10}heterocycloalkyl; C_5-C_{10}heteroaryl; nitro; C_2-C_8alkylcarbonyl; $-N(C_1$-C_8alkyl$)C(O)$ C_1-C_8alkyl; $-SO_2N(R^{10})R^{11}$; $-N(R^{10})R^{11}$; $-C_1$-C_4-alkylene-$SO_2N(R^{10})R^{11}$; $-CON(R^{10})R^{11}$; wherein each of R^{10} and R^{11} independently is hydrogen; hydroxy; C_1-C_8alkyl; C_2-C_8alkenyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkyl-C_1-C_8alkyl; C_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkoxyC_1-C_8alkyl; hydroxyC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl which optionally may be substituted on the ring by hydroxy, C_1-C_8alkoxy, carboxy or C_2-C_8alkoxycarbonyl; thiazolyl; or pyridylalkyl; or R^1 and R^2 form together with the C-atoms to which they are attached a 5 to 10 membered aryl or heteroaryl residue, the latter comprising one or two heteroatoms selected from N, O and S; or

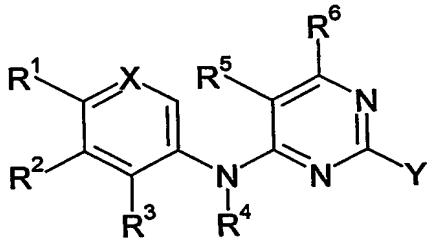
each of R^5 and R^6 independently is hydrogen; halogen; cyano; C_1-C_8alkyl; C_2-C_8alkenyl; C_2-C_8alkynyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkylC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl;

each of R^7, R^8 and R^9 independently is hydrogen; hydroxy; substituted or unsubstituted 5 or 6 membered heterocyclic ring comprising 1, 2 or 3 heteroatoms selected from N, O and S; C_1-C_8alkyl; C_2-C_8alkenyl; C_3-C_8cycloalkyl; C_3-C_8cycloalkylC_1-C_8alkyl; C_5-C_{10}arylC_1-C_8alkyl; C_1-C_8alkoxy; $-CF_3$; $-CONR^{10}R^{11}$; $-N(R^{10})(R^{11})$; C_3-C_8cycloalkylC_1-C_8alkyl; heteroC_3-C_8cycloalkyl; carboxy; C_1-C_8alkoxycarbonyl; $-SO_2N(R^{10})R^{11}$; halogenoC_1-C_8alkyl; or R^7 and

R^8 are forming together with the carbon atoms to which they are attached, a 5 or 6 membered heteroaryl radical comprising 1, 2 or 3 heteroatoms selected from N, O and S; in free base or salt form.

2. A compound of claim 1 wherein X is "=CR^0-", R^0, R^1, R^4, R^5, R^6 = H; R^2 = H or CH_3 or methoxy; R^3 = SO_2NH_2; R^7 and R^8 is bridged by -NH-N=CH- or each methoxy; and R^9 is H or methoxy.

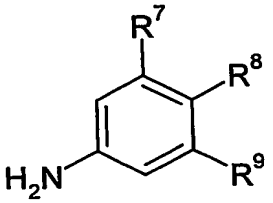
3. A process for the production of a compound of formula I according to claim 1, comprising the steps of reacting a compound of formula II



(II)

wherein R^1, R^2, R^3, R^4, R^5, R^6 and X are as in claim 1, and Y is a leaving group;

with a compound of formula III



(III)

wherein R^7, R^8 and R^9 are as defined in claim 1;

and recovering the resulting compound of formula I in free or in form of a salt, and, where required, converting the compound of formula I obtained in free form into the desired salt form, or vice versa.

4. A compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, for use as a pharmaceutical.

5. A compound of claim 1 in free base or pharmaceutically acceptable acid addition salt form, for use in the treatment or prevention of a disease or condition in which ZAP-70 and/or Syk tyrosine kinase activation plays a role or is implicated.

6. A pharmaceutical composition comprising a compound of claim 1 in free base or pharmaceutically acceptable salt form, in association with a pharmaceutical carrier or diluent.

7. The use of a compound of claim 1 in free base or pharmaceutically acceptable salt form, as a pharmaceutical for the treatment or prevention of a disease or condition in which ZAP-70 and/or Syk tyrosine kinase activation plays a role or is implicated.

8. A combination which comprises (a) a therapeutically effective amount of a compound of claim 1 in free base or pharmaceutically acceptable form and (b) a second drug substance.

9. A method for treating or preventing a disease or condition in which ZAP-70 and/or Syk tyrosine inhibitor activation plays a role or is implicated, in a subject in need of such treatment, which comprises administering to such subject a therapeutically effective amount of a compound of claim 1 in free base or pharmaceutically acceptable salt form.

10. A method according to claim 9, wherein the compound of claim 1 is administered in combination with a second drug substance.